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Use of the promoter fusion transposon Tn5 lac to identify mutations in Bordetella pertussis vir-regulated genes.

Weiss AA, Melton AR, Walker KE, Andraos-Selim C, Meidl JJ.

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Mutants of Bordetella pertussis deficient in virulence-associated factors were identified by using the transposon Tn5 lac. Tn5 lac is a derivative of Tn5 which generates promoter fusions for beta-galactosidase. Tn5 lac insertions in the vir-regulated genes of B. pertussis were identified by selecting for kanamycin-resistant mutants that expressed beta-galactosidase when the vir-regulated genes were expressed but not when the vir-regulated genes were turned off. Fourteen different mutations in vir-regulated genes were identified. Two mutants were deficient in the production of the filamentous hemagglutinin, two mutants were deficient in the production of adenylate cyclase toxin and hemolysin, and one mutant was deficient in the production of dermonecrotic toxin. One insertion mapped adjacent to the pertussis toxin gene, but the mutant produced pertussis toxin. The phenotypes of the remaining eight mutants were not determined, but the mutants did not appear to be deficient in the production of the 69,000-dalton outer membrane protein (agglutinogen 3) or the capsule. Screening for mutations in either of the fimbrial genes proved to be problematic since the parental strain was found to switch from a fimbriated to a nonfimbriated state at a high frequency, which was suggestive of the metastable expression of pili in other bacteria. We used Southern blot analysis with a 30-mer specific for the fimbrial sequences. No bands with the predicted increase in size due to the 12 kilobases from Tn5 lac were observed, which suggests that none of these genes were mutated. Southern blot analysis also revealed that seven of the eight unidentified mutations mapped to different restriction fragments, which suggests that they could be deficient in as many as seven different genes.

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The role of cysteine 41 in the enzymatic activities of the pertussis toxin S1 subunit as investigated by site-directed mutagenesis.

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The S1 subunit (Mr 28,000) of pertussis toxin expresses thiol-dependent enzymatic ADP-ribosyltransferase and NAD-glycohydrolase activities. Site-directed mutagenesis experiments were performed on the codon for Cys-41 of this subunit to investigate the role of this residue in both enzymatic activities. Deletion of Cys-41 caused a decrease in both activities below detectable levels, whereas replacement of this residue by serine, glycine, proline, or asparagine only slightly reduced the activities. The enzymatic activities of these mutants were thiol-independent. The deletion of Ser-40, adjacent to Cys-41, again caused reduction of the enzymatic activities to undetectable levels. Steady-state kinetic experiments showed that the kcat of the mutant protein in which Cys-41 was replaced by glycine was nearly identical to the kcat of the parent version. However, the Km for NAD of the mutant was significantly higher relative to that of the wild type version. These results indicate that the side-chain of Cys-41 is not essential for enzymatic activities and that Cys-41 is not involved in the rate of catalysis but is probably located at or close to the NAD-binding site. The introduction of a negative charge at position 41 through the replacement of Cys-41 by either aspartate or glutamate reduced the enzymatic activities to very low but measurable levels, suggesting a charge-charge repulsive interaction between these residues and possibly one or both of the phosphates of NAD. Cys-41 may therefore be located close to the phosphate subsite of the NAD-binding site.

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